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An Interpretation of Some Differences in the Percentage of Water Found in the Central Nervous System of the Albino Rat and Due to Conditions Other than Age.

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AN INTERPRETATION OF SOME DIFFERENCES IN THE PERCENTAGE OF WATER FOUND IN THE CENTRAL NERVOUS SYSTEM OF THE ALBINO RAT AND DUE TO CONDITIONS OTHER THAN AGE

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There are now available ten different series of observations bearing on the problem of variation in the percentage of water in the brain and spinal cord of the albino rat when subjected to unusual conditions. Nine of these series have been already published. The differences or variations which have been found are in most cases slight, in one instance only being more than 0.5 per cent. Differences of this order would usually be classed as errors of observation, but the regularity of their occurrence in some instances leads me to think that at times they are really significant. The present paper gives a brief survey of the results so far obtained, noting those cases in which the differences, though slight, seem to be significant, and attempting to offer an explanation for them. As the investigations which will be discussed are, with one exception, already in print, it will be unnecessary to repeat in the present paper many details which have been already given in the published articles.

In the first instance attention is called to a difference in the percentage of water which is mainly of technical interest and which depends on the effect of the interval between death and dissection. This matter has been studied by Dr. King ('11).

As will be seen by referring to her paper (p. 152) healthy rats dissected eight hours after death show an excess of 0.3 per cent of water in the brain. On the other hand, rats killed when suffering from pneumonia and remaining untouched for about the same time before dissection show from 0.3 to 0.4 per cent more water than is found in normal animals, and 0.7 to 0.8 per cent more than is found in "pneumonia" rats dissected immediately after killing. This last group of course is the one with which they should really be compared. This result serves to emphasize the necessity of immediate or early dissection, when accurate determinations of the percentage of water are desired.

I am not aware that this post-mortem absorption of water has been previously studied in mammals, but the results are in accord with earlier findings in the brain and spinal cord of the frog, Donaldson ('98), p. 318; Donaldson and Schoemaker ('02).

The change is probably due to the taking up of water from the surrounding media as is indicated by the smaller amount of the increase in "dry" frogs—frogs namely in which the amount of water in the entire animal has been reduced below the normal. (Donaldson and Schoemaker, '02.)

The attempt has sometimes been made to determine in man whether the percentage of water in the nerve tissues has been changed by certain diseases (*e.g.*, Weisbach, '68). As, however, the effects of the post-mortem interval have never been either eliminated or controlled in these observations, the results must be considered as vitiated in a measure by an unknown factor.

Nine other investigations remain to be examined. These may be grouped under two general headings:

(A) Investigations in which the growth of the body as a whole, or of the central nervous system alone, or both together, was going on with more than normal vigor, either at the time of death or at some previous period in the life of the rat.

(B) Investigations in which the corresponding growth processes had been either arrested or carried on with less than normal vigor.

I shall present and discuss in the first place the investigations which form group (A). In this first group (A) there are to be found five studies dealing:

(1) With the influence of exercise; (2) with the influence of the bearing of young; (3) with the effect of severe underfeeding for a short time—followed by a return to the normal diet; (4) with prolonged underfeeding—followed by a short period on the normal diet; (5) with the effect of spaying.

(1) It has been shown (Donaldson, '11) that the effect of exercise, as obtained by a rat living in a revolving cage, is to slightly retard growth in body length without modifying the growth in body weight, and at the same time to cause an increase of 2.5 to 2.7 per cent in the weight of the brain, while leaving the weight of the spinal cord unaffected.

The percentages of water observed in the brain and spinal cord are as follows:

TABLE 1

	BRAIN	SPINAL CORD
February series		
Exercised	78.514	71.886
Controls	78.517	72.203
Exercised	−.003	−.317
October series		
Males: Exercised	78.372	70.961
Males: Controls	78.317	70.978
Males: Exercised	+.055	−.017
Females: Exercised	78.471	71.251
Females: Controls	78.390	71.172
Females: Exercised	+.081	+.079
Average difference in exercised: all groups	+.044	−.085

The table shows a very slight average excess in the percentage of water, .044 in favor of the brains from the exercised rats, while in the case of the spinal cords from the exercised rats there is an average deficiency of .085 per cent.

While the excess of the percentage of water in the brain might be connected with the slightly greater brain weight in the exercised group, there is no corresponding deficiency in the weight of the spinal cord to explain the deficiency of water found in it. As the matter stands then, the data do not permit of any conclusion except that the percentage of water in the central nervous system is not definitely modified in exercised rats.

(2) Watson ('05) found that rats bearing young, as contrasted with unmated rats of the same litters, had a somewhat greater body weight, a brain weight which was the same, but a distinctly heavier spinal cord, while the percentage of water on the average was as follows:

	BRAIN	SPINAL CORD
Mated	77.47	68.51
Unmated	77.37	68.29
Difference, mated	+0.10	+0.22

In commenting on these relations it can be said at the outset that there is no question that it is characteristic of mated animals to have a slightly greater percentage of water in the central nervous system, for it occurs in nine out of the ten comparisons which can be made with these data—but how it comes about is not so clear. There is no conclusive evidence that the mated rats have heavier brains, yet they do have 0.1 per cent more water in the brain. On the other hand, with the distinctly heavier cords of these rats goes 0.22 per cent, more water. Also the mated rats have about 9 per cent more body weight. In this case therefore the greater amount of water in the central nervous system can be most simply related to the more vigorous growth of the body as a whole.

(3) Hatai ('07) found that the effect of severe underfeeding for twenty-one days, followed by a return to the normal diet, yielded, at two hundred days, animals of nearly the same weight as the controls; indeed the rats which had been subjected to underfeeding were on the average a little heavier than the controls, while the weight of the central nervous system was not modified by this treatment.

On the other hand, the percentages of water in both the brain and spinal cord were found to be greater in the animals subjected to experiment, and this was true not only of the general averages, but also the detailed comparisons by pairs. The following table gives the average values for the percentages of water:

TABLE 2

	BRAIN	SPINAL CORD
Males		
Experimented	77.75	70.05
Controls	77.50	69.71
Difference in the experimented	+0.25	+0.34
Females		
Experimented	77.75	70.10
Controls	77.50	69.40
Difference in the experimented	+0.25	+0.70
Average for both sexes	+0.25	+0.52

In this instance also there was no increase in the weight of the central nervous system in the experimented animals with which to associate the greater percentage of water, but the latter must be connected with the more vigorous growth processes which occurred after these animals had been returned to a normal diet, as indicated by the fact that they overtook, and in the case of the males actually surpassed, the controls in body weight.

(4) In a later study, Dr. Hatai ('08) followed the effects of a very meager diet on five litters of rats until they were from three to six months of age. These animals were then returned to the normal diet for thirty days and at the end of that time were killed. The body weight was of course much reduced by this treatment and the animals were stunted, and when killed were found to be short for their body weight. Nevertheless the weight of the central nervous system was normal to the body weight. When the percentage of water in the brain and spinal cord was determined, however, it was found to be as follows:

	BRAIN	SPINAL CORD
Stunted	78.609	72.565
Controls	78.383	71.224
Difference in favor of the stunted rats	+0.226	+1.341

Again we find the greater percentage of water in the brain and spinal cord of the stunted rat, yet in relation to the body weight, the weights of these organs were normal.

The prime difference between the stunted rats and the controls, when both were killed, lay in the revival of growth activity in the stunted as a result of the return to the normal diet for thirty days. Owing to the relative shortening of the body in the stunted, such activity might be thought of as greater in the spinal cord than in the brain, and with this greater activity could be associated the larger difference in the percentage of water in the spinal cord. In brief, then, we associate the greater percentage of water in the central nervous system of the stunted rats with a revival of the general growth processes following the return to the normal diet.

(5) For this presentation Dr. Stotsenburg permits me to use some of his unpublished data on the effect of spaying the female albino rat.

Following this operation, as Dr. Stotsenburg has shown, the spayed rats become somewhat larger and fatter than the controls, but the relative weights of the brain and spinal cord are not apparently modified. For the percentage of water, four litters are at present available and the values for the percentage of water in the spayed and control groups of each of these litters is given in table 3.

An examination of table 3 shows that the percentage of water in the brain and in the spinal cord is not modified by the operation of spaying.

Although the body growth of the spayed rat has been increased by the operation, the difference in the vigor (rate) of growth at the time when these animals were killed was very slight, and this

TABLE 3

Percentage of water in the brain and spinal cord of spayed as compared with control rats. Eleven spayed—ten controls

LITTER	AGE IN DAYS	PERCENTAGE OF WATER IN BRAIN		PERCENTAGE OF WATER IN SPINAL CORD	
		Spayed	Control	Spayed	Control
7	139	78.940	78.773	72.396	72.217
5	259	78.083	78.168	71.489	71.487
4	291	78.114	78.102	70.286	70.544
3	427	77.382	77.396*	69.258	69.533*
Average		78.129	78.110	70.857	70.945

* The control in the third litter was killed at 384 days of age, while the spayed were killed at 427 days. The percentage of water in the control brain at 384 days was 77.482.

I have calculated, however, that between that age and 427 days, the brain was losing water at the rate of .002 per cent per diem. The correction for the intervening 43 days was therefore made and the value, 77.396, given in the table, was that found.

Similarly, I calculated that during the 43 days in question the spinal cord was losing water at the rate of .0065 per cent per diem, and accordingly the observed percentage, 69.813 was corrected to 69.533, the figure given in the table.

seems the probable reason for the similarity of the percentage of water in the two groups.

The foregoing series of five investigations constitute group (A). Further comment, however, on the findings in this group will be deferred until the investigations which form group (B) have been presented.

Group (B) is represented by four investigations in which growth has been either arrested or retarded. This has been brought about (6) by underfeeding; observations by Dr. Hatai, (7) by underfeeding; observations by myself, (8) as the result of pneumonia, (9) by castration, which, although it does not affect the body growth, does retard slightly the growth of the brain.

(6) In 1904 Hatai ('04) by underfeeding was able to reduce the weight of the brain in rats of his first series so that at the end of the twenty-one days the brains of the underfed rats weighed 12.8 per cent less than those of the controls killed at the same time. By this treatment also the body weight was reduced to 44 per cent

of the controls. When the brains were examined for the percentage of water, the following was found:

Percentage of water in the controls	79.11
Percentage of water in the underfed	78.91
Deficiency in the underfed	0.20

In this case both the brain as well as the body as a whole lost in weight and this arrest of growth is the probable cause of the loss in water indicated above.

In rats of Hatai's second series, the controls were killed at the *beginning* of the twenty-one days during which the other rats were underfed. Here there was a corresponding loss in both brain weight and body weight similar in amount to that found in the first series. The determination of the water in the brain showed

Percentage of water for the control rats	79.01
Percentage of water for the underfed rats	78.71
Deficiency in the underfed	0.30

The greater difference or deficiency shown by the underfed rats in this second series is due to the fact that they were twenty-one days *older* than the controls which were killed at the beginning of the experiment, and hence in the case of the percentage of water, the amount lost as the result of advancing age is added to the amount lost on account of the arrest of growth.

These results of Hatai have been reviewed and revised in a foregoing paper (Donaldson, '11). As it is not possible to separate the influence of the loss of brain weight from that of the loss of body weight, we are able to conclude only that when the nutritive conditions cause such losses, the percentage of water in the brain is slightly reduced.

(7) My own observations (Donaldson, '11) were made on a much larger series of litters, but the animals were younger and the nutritive disturbance was not so severe.

As a result, the underfed rats at the end of twenty-one days had a body weight but slightly below that at the beginning of the experiment—while the weight of both the brain and the spinal cord increased somewhat, although in both of these organs the growth was retarded. The following gives the percentages of water found:

	BRAIN	SPINAL CORD
Percentage of water in controls	79.394	73.985
Percentage of water in underfed	79.278	74.032
Difference in underfed	−0.116	+0.047

From the manner in which it occurs (*loc. cit.*, table 2) the difference in the percentage of water in the spinal cord is probably not significant, so that we shall deal only with the difference which appears in the brain. This occurs while the brain is still growing slightly and is only about half as great a difference as that found by Hatai in his series in which, however, the nutritive disturbance was much more severe.

We conclude therefore that underfeeding which stops the growth of the body and retards that of the nervous system, does not modify the percentage of water in the spinal cord, while it does reduce it in the brain—the amount of this reduction being less in the cases where the underfeeding is less severe.

(8) In the study of the effect of pneumonia on the weight and other characters of the central nervous system, Dr. King ('11) found that in the rat, pneumonia, which causes a marked loss in body weight and at least a retardation in the increase of the weight of the brain, produces in the brain a very distinct loss in the percentage of water.

Dr. King's table 4 is here repeated to show this:

TABLE 4

NUMBER OF CASES	PERCENTAGE OF WATER		DEFICIENCY OF WATER PNEUMONIA RATS
	Observed	Normal Calculated	
13 males	77.68	78.19	0.51
19 females	77.61	78.04	0.43

There here appears a deficiency of 0.4 to 0.5 per cent in the water of the brain in rats exhibiting well marked lesions of pneumonia. This is the largest deficiency thus far found. These rats have in common with those just described (B, 6 and 7) a loss in body weight and arrest in the growth of the brain and doubtless some of the deficiency can be credited to these changes, but according to what we have observed in the foregoing investigations they would account for a fraction only of the deficiency. Hence we infer that in some way "pneumonia" aids to decrease materially the percentage of water in the brain. Decrease in the percentage of water in the human brain after diseases causing general wasting of the body has been found by Weisbach ('68) and others. These diseases must be distinguished from such as general paralysis, where there is wasting of brain substance accompanied by an increase in the percentage of water (Barratt, '00) (Koch and Goodson, '06).

(9) The observations of Dr. Stotsenburg and of Dr. Ranson, published by Stotsenburg ('09) show that castration does not modify body growth in the albino rat. Observations by Dr. Hatai and myself (Donaldson and Hatai, '11) indicate that both the brain and spinal cord are arrested in their growth by this operation, so that they remain permanently smaller than in the controls.

At the same time, if we take the averages of the percentages of water from the three tables of the foregoing paper (Donaldson and Hatai, '11) we obtain the following:

TABLE 5

Percentage of water

	BRAIN		SPINAL CORD	
	Castrated	Control	Castrated	Control
Table 1	77.42	77.36	68.64	68.29
Table 2	77.48	77.54	68.77	68.80
Table 3	77.94	77.99	69.68	69.99
Average	77.61	77.63	69.03	69.03

As will be seen by the examination of table 5 there is not the slightest evidence that the retardation in the growth of the brain and spinal cord in the castrated rats is accompanied by a modification of the percentage of water in these organs. It follows from this that conditions which cause a slight retardation in the growth of the central nervous system alone are not necessarily accompanied by variations in the percentage of water.

To summarize the results presented in group (B) we observe that underfeeding more or less severe causes a corresponding deficiency of water in the brain, that "pneumonia" causes a very marked deficiency, while castration, although it arrests somewhat the growth of the brain and spinal cord, does not modify the percentage of water in these organs.

If we contrast the two groups (A) and (B) as to the percentage of water in the central nervous system, we find in group (A) that when active body growth is in progress and the anabolic processes are vigorous, we have an increase in the percentage of water (2) in the brain and spinal cord in the investigations (2), (3) and (4).

In the investigations (1), influence of exercise, and (5), the influence of spaying, in which the body growth changes are slight, no clearly marked effects on the percentage of water were observed.

On the other hand, in group (B) where growth in the body as a whole, or in the central nervous system in particular, had been more or less retarded and even loss of weight had occurred, a loss in the percentage of water was found in (6) in the brain (spinal cord not examined); in (7) in the brain—spinal cord not affected; in (8) pneumonia; in the brain—spinal cord not examined—while in (9) castration, no influence on the percentage of water was found. These results can be formulated in the following conclusions:

1. The changes in the percentage of water in the central nervous system of the albino rat, which can be induced experimentally, are usually less than 0.5 per cent in amount.

2. They depend more on the metabolic activities of the body as a whole, than on those of the nervous system alone.

3. When anabolic processes predominate, the percentage of water tends to increase.

4. When catabolic processes predominate, the percentage of water tends to decrease.

The means by which these changes are mainly brought about are possibly two—namely variations of the amount of blood in the brain and variations in the amount of water in the cell bodies of the neurones.

Following first the suggestion that these results may be due in part to variations in the amount of blood, the facts to be presented are these:

The percentage of water in the blood of the albino rat is about 80.0 per cent as recently determined in this laboratory.

This is high when compared with the findings in the case of man, where it is 78.10 per cent for the male; 79.37 per cent for the female, as determined from the table in Vierordt ('06) by taking the average of the last six entries.

Ranke ('71) gives the fraction of the total amount of blood found in the brain and spinal cord of the rabbit as 1.24 per cent, and of this one per cent would be a liberal assignment to the brain. The relations of body weight and brain weight in the rabbit are near enough alike to warrant the assumption that we should have the same relations in the rat, that is 1.0 per cent of the total weight of the blood in the brain. On the basis of the determinations by Jolly and Stini ('05) we consider 4.5 per cent of the total body weight of the albino rat to be represented by the weight of the blood. In a rat having a body weight of 200 grams, this would give 9 grams of blood, and according to the previous argument 1.0 per cent of this, or .09 grams, would be the quantity of blood in a brain weighing 1.85 grams, which is approximately the weight of brain found in the rat of the size chosen. The brain would then contain about 4.8 per cent of its weight in the form of blood.

In an entire brain having 78 per cent of water and containing 4.8 per cent of blood in which the percentage of water was 80.0 per cent, any variations in the amount of blood would of course produce alterations in the percentage of water; an excess of blood causing the percentage to rise and a deficiency of blood causing it to fall. Calculations show that it would be necessary to double the normal amount of blood in the brain (*i.e.*, raise it to 9.6 per cent) to cause a gain of 0.1 in the percentage of water in the entire brain, and conversely to reduce the amount of blood to zero to cause a corresponding loss of 0.1 per cent.

This, of course, is an almost impossible range in the quantity of the blood, but it is in the right sense, for vigorous anabolic processes might be expected to increase the supply of blood to the brain and thus raise the percentage of water, and conversely, excessive catabolism might be expected to give the contrary result, with a fall in the percentage of water. If, however, we consider the changes in the quantity of blood in the brain as moderate instead of extreme, then, although the variations in the blood supply are in harmony with the changes found, yet they would hardly be adequate to explain the entire differences actually observed. To complete the explanation therefore, it is necessary to look to variations in the neurones themselves.

Naturally we should think of these variations as taking place in the cell bodies. There is reason to conclude that in man the cell bodies of the neurones form only about two per cent of the total weight of the encephalon (Thompson, '99).

There is also reason to conclude that in the encephalon of the rat the relative weight of the cell bodies to the entire mass is likewise about 2 per cent. If then the alteration of the percentage of water in the brain were due in part to changes in the volume (weight) of the cell bodies, and these formed but 2 per cent of the entire mass, a variation of 0.1 in the percentage of water would mean a change of 5 per cent in the cell bodies, which would be an alteration of some significance. Thus an important variation in the amount of water in the cell bodies would affect the percentage of water in the entire brain to only a very slight degree.

The suggestion that the cell body is the portion of the neurone where the changes in percentage take place most easily, and the recognition of the relatively small proportion of the encephalon represented by cell bodies, serves to explain, in conjunction with what has been said about the variations in the quantity of blood, how it is that we can have small differences in the percentage of water which under given conditions occur with such regularity, and how differences in the percentage of water as determined for the entire encephalon which are absolutely so small, may yet have a significant physiological value when referred to the two causes just mentioned.

The fundamental change in the percentage of water in the central neurones is that due to age (Donaldson, '10) and it does not seem possible to significantly modify this slow, steady diminution which is a function of time, except for short periods and under abnormal conditions such as were produced by Engels ('03–'04) in his study on the absorption of water. We have just seen, however, that there is probably a small amount of variable water, if such a term may be used, which can come and go in the cell, and which undoubtedly has some value for the functions of the cell, but which must always be distinguished from the fixed water which is more intimately linked with cell constituents and not subject to this sort of flux.

We conclude therefore that the variations in the percentage of water under discussion have been caused (1) by alterations in the quantity of blood in the brain and (2) by changes in the amount of variable water in the cell bodies of the neurones, but the relative importance of the two factors cannot at the moment be determined.

The practical suggestions from the foregoing which are of use for the methods of study and technique and which should be regarded in order to obtain results on the percentage of water in the brain that can be used for comparison, are as follows:

1. The age of the animal must be known and the younger the animal the more precise should be the age determination.

2. The brain should be removed from the body within an hour or two after death. See influence of post-mortem interval.

3. Brains of individuals dying from chronic diseases should be excluded as they will probably have abnormal percentage of water. See pneumonia rats.

4. The ordinary variations in the nutritive conditions of animals may be disregarded as they will not produce any marked variation in the percentage of water in the brain.

It is hardly necessary to add that the human brains used for studies on the percentage of water rarely comply with conditions (2) and (3) as given above.

LITERATURE CITED

Barratt, W. 1900 On the amount of water and phosphorus contained in the cerebral hemispheres and spinal cord in general paralysis of the insane and in other conditions. Archives of Neurol. from the Path. Lab. of the London County Asylums, vol. 1, pp. 207–223.

Donaldson, H. H. 1898 Observations on the weight and length of the central nervous system and of the legs, in bullfrogs of different sizes. Jour. Comp. Neur., vol. 8, no. 4, pp. 314–335.

1911 The effect of underfeeding on the percentage of water, on the ether-alcohol extract, and on medullation in the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, no. 2.

1911. On the influence of exercise on the weight of the central nervous system of the albino rat. Jour. Comp. Neur., vol. 21, no. 2.

Donaldson, H. H., and Schoemaker, D. M. 1902 Observations on the postmortem absorption of water by the spinal cord of the frog (Rana virescens). Jour. Comp. Neur. Psych., vol. 12, no. 2, pp. 183–198.

Donaldson, H. H., and Hatai, S. 1911 Note on the influence of castration on the weight of the brain and spinal cord in the albino rat and on the percentage of water in them. Jour. Comp. Neur., vol. 21, no. 2.

Engels, W. 1903, 1904. Die Bedeutung der Gewebe als Wasserdepots. Arch. Exp. Pathol. und Pharmacol., vol. 51, pp. 346–360.

Hatai, S. 1904 The effect of partial starvation on the brain of the white rat. Am. Jour. Physiol., vol. 12, no. 1, pp. 116–127.

1907 Effect of partial starvation followed by a return to normal diet, on the growth of the body and central nervous system of albino rats. Am. Jour. Physiol., vol. 18, no. 3, pp. 309–320.

1908. Preliminary note on the size and condition of the central nervous system in albino rats experimentally stunted. Jour. Comp. Neur. Psych., vol. 18, no. 2, pp. 151–155.

Jolly, J., and Stini, J. 1905 Masse totale du sang chez le rat blanc. C. R. Soc. Biol., Paris, vol. 58, pp. 835–837.

King, Helen D. 1911 The effects of pneumonia and of postmortem changes on the percentage of water in the brain of the albino rat. Jour. Comp. Neur., vol. 21, no. 2.

Koch, Waldemar, and Goodson, W. H. 1906. A preliminary study of the chemistry of nerve tissue degeneration. Am. Jour. Physiol., vol. 15, pp. 272–279.

Ranke, J. 1871 Tetanus. Eine Physiologische Studie. vol. 2, 8°, Leipzig, W. Engelmann. Hft. 2. Die Blutvertheilung und der Thätigkeitswechsel der Organe, etc., pp. 80 and 81.

Stotsenburg, J. M. 1909 On the growth of the albino rat (Mus norvegicus var. albus) after castration. Proc. of the Assoc. of Am. Anat., Anat. Rec., vol. 3, no. 4, pp. 233–244.

Thompson, Helen B. 1899 The total number of functional cells in the cerebral cortex of man, and the percentage of the total volume of the cortex composed of nerve cell bodies, calculated from Karl Hammarberg's data. Jour. Comp. Neur., vol. 9, no. 2, pp. 113–140.

Vierordt, H. 1906 Daten und Tabellen für Mediziner. Dritte Auflage. Gustav Fischer, Jena.

Watson, J. B. 1905 The effect of the bearing of young upon the body weight and the weight of the central nervous system of the female white rat. Jour. Comp. Neur. Psych., vol. 15, no. 6, pp. 514–524.

Weisbach, A. 1868 Der Wassergehalt des Gehirns nach Alter, Geschlecht und Krankheiten. Med. Jahrbücher, vol. 16, nos. 4 and 5, pp. 1–76.